Title: Assessing vascularization capacity and barrier properties of BBB-specific endothelial cells and pericytes

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Abstract

Luminal layer of blood-brain barrier (BBB) is formed by specialised brain vascular endothelial cells (BMECs). BBB assembly and barrier function depend on vascular supporting pericytes (PC) forming its outer layer. In diseases affecting BBB integrity, interaction between BMECs and PCs is often altered.

As induced pluripotent stem cell-derived brain endothelial-like cells (hiPSC-BMECs) are an attractive approach in BBB in vitro modeling, we aim to differentiate hiPSC-BMECs and study their vascularisation capacity with different pericytes.

We established the differentiation protocol for hiPSC-BMECs and immunocytochemically characterized them for endothelial and BBB markers. Functionality of the hiPSC-BMECs was tested with 2D vasculogenesis assay with three different peripheral mural cell types. Of these, bone marrow stem/stromal cells (BMSCs) were compared to brain-specific mural cells (human primary brain pericytes, pBPCs) to assess vascular network formation in 2D and 3D co-cultures. Also, transendothelial electrical resistance (TEER) was measured in 2D co-culture. To assess the brain-specificity of the hiPSC-BMECs, human umbilical vein endothelial cells (HUVECs) were included in the study as a control.

Immunocytochemistry showed BMECs express some of the BBB markers in varying degree. While the first 2D vascular formation tests with the three initial mural cell types, especially BMSCs, showed potential, pBPCs´ ability to support BMECs´ vascular formation in 2D was modest. In 3D, pBPCs were not capable of acting as vascular supporting cells. Contrarily, TEER values were improved in pBPCs + hiPSC-BMEC coculture suggesting an improved barrier formation. These preliminary results are promising but highlight the complexity of developing tissue-representative in vitro BBB model.